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Performance of groundnut (*Arachis hypogaea* Linn) under nitrogen fixing and phosphorus solubilizing microbial inoculants with different levels of cobalt in alluvial soils of eastern India

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Abstract. A field experiment was conducted for three years to evaluate the performance of groundnut under alluvial soil of eastern India with different types of inoculants such as *Rhizobium* and phosphobacterium inoculants, no inoculant, and different levels of cobalt (0.21 and 0.42 kg ha^{-1}). Results indicated that *Rhizobium* inoculant promoted higher yield and nutrient uptake as compared to phosphobacterium. Kernel yield, averaged across three levels of cobalt, was recorded to be highest for *Rhizobium* inoculant, which was 16.50% and 10.72% higher over no inoculant and phosphobacterium inoculant respectively. Cobalt at the rate 0.21 kg ha^{-1} proved to be better as compared with other doses of cobalt and resulted in 10% higher kernel yield over no cobalt application. Among different treatment combinations, integrated application of *Rhizobium* + cobalt at the rate 0.21 kg ha^{-1} resulted in greater yield than that of other combinations, followed by phosphobacterium inoculant with the same dose of cobalt application. The yield and uptake of N, P and K by groundnut was significantly higher in the treatments receiving both inoculants and cobalt applied at 0.21 kg ha^{-1} than individual application of either inoculants or cobalt. The beneficial effect of application of microbial inoculants and cobalt was also reflected on the soil fertility status.

Key words: *Arachis hypogaea*, *Rhizobium*, phosphobacterium, cobalt, kernel yield

INTRODUCTION

India is blessed with the agro-ecological condition favorable for growing nine major oilseeds including seven edible oilseeds, namely groundnut, rapeseed-mustard, soybean, sunflower, safflower, sesame and niger and two non-edible sources, namely castor and linseed, apart from a wide range of other minor oilseeds and oil-bearing tree species. Among all the oilseed crops, peanut occupies the first place in India accounting for more than 28% of acreage and 32% of production in the country (Anonymous, 2004). However, except for castor, the productivity of oilseed crops in India is one of the lowest in the world.

It has been reported that low yield in groundnut is probably due to low nodulation due to competition from strains in the soil which are ineffective with this host. Nodules formed by the native strains may not be able to fix sufficient nitrogen to meet the demand of the plant. But recent studies showed that *Rhizobium* inoculant has a favorable effect on legumes like groundnut (Joshi et al., 1989). This inoculant helps to meet the additional nitrogen demand of the plant, by increasing nodulation, enabling realization of the yield potential of the plant.

Phosphorus plays an important role in nodulation of legume crops. Phosphobacterium, a phosphate solubilizing bacteria, able to convert the phosphate present in the soil from unavailable to an available-to-the plant form, has an indirect but definite effect on the nodulation and yield of legume crops like groundnut. Phosphate solubilizing bacteria improve nodulation (Ghosh & Poi, 1998) through increased phosphate solubilization and hence, increase symbiotic nitrogen fixation (Dametario et al., 1972).

Cobalt increases nodule formation (Reddy & Raj, 1975). Many workers isolated the cobalamine coenzyme B_{12} from the root nodules of legumes and non-legumes, and pointed out the interdependence of the cobalt supply, the B_{12} co-enzyme content of *Rhizobium,* the formation of leghemoglobin, and nitrogen fixation (Das, 2000). It has now been established that *Rhizobium* and other nitrogen-fixing microorganisms have an absolute cobalt requirement. Three specific cobalamine dependent enzyme systems in *Rhizobium* which may account for the influence of cobalt on nodulation and nitrogen fixation are: Methionine synthase**,** Ribonucleotide reductase and Methylmalonyl co-enzyme A mutase (Das, 2000). However, information on the effect of cobalt, *Rhizobium*, a nitrogen fixer and phosphobacterium, a phosphate solubilizer, on groundnut is lacking (Reddy & Raj, 1975; Jana & Sounda, 1994). Hence the experiment was conducted to study the effect of cobalt, *Rhizobium* and phosphobacterium in combination on yield, oil content and nutrient uptake by groundnut.

MATERIALS AND METHODS

The experiment was conducted during kharif season (mid-June to mid-October), 2001, 2002 and 2003 at Gangetic alluvium soil (Entisol) of West Bengal, India. The soil characteristic of the experimental site was sandy clay loam with neutral pH (7.2). The organic carbon, available N, P, K and Co content was 0.63%, 103.2 mg kg^{-1}, 20.1 mg kg^{-1}, 88.3 mg kg^{-1} and 0.03 mg kg^{-1}, respectively. The weather of the experimental site was warm and humid.

Three types of inoculants viz., inoculation with either *Rhizobium* (I_R) and phosphobacterium (I_P) or no microbial inoculants (I_0) and three levels of cobalt viz., no cobalt (C_0), 0.21 kg ($C_{.21}$) and 0.42 kg cobalt ha^{-1} ($C_{.42}$) were studied. Inoculants were allotted to main plots and levels of cobalt to the sub plots under each main plot. There were nine treatment combinations (three inoculants x three levels of cobalt) laid out in a split plot design with three replications. Cobalt was mixed with soil in granular form as cobalt sulfate ($CoSO_4$, $7H_2O$). The *Rhizobium* strain was M-10 and the phosphobacterium strain was *Bacillus polymyxa*, obtained form Nodule Research Laboratory, West Bengal, India. Inoculants were lignite based and applied by inoculating the seed before sowing. Groundnut received 20 kg N, 40 kg P_2O_5 and 20 kg K_2O ha^{-1} in the form of urea, single super phosphate and muriate of potash respectively as basal. Groundnut (cv. AK-12-24) was sown in line with spacing of 30 cm x 10 cm during the second week of June. The crop was harvested at 120 days after sowing. The plot size was 16 m^2 (4 m x 4 m). At harvest observations were taken on yield, oil content, nutrient uptake and residual soil fertility.

Yield components viz., number of pods plant^{-1} and number of kernels pod^{-1} were recorded from 10 plants randomly selected. For determining test weight, 100 completely-filled kernels were randomly counted, weighed and multiplied by 10. Total harvested biomass from one square meter area leaving two border rows on all sides was weighed after sun drying. Pods were separated from haulms and weighed; haulm yield was determined by subtracting pod yield from total biomass yield. Pods were unshelled to get the kernel yield.

Chemical analysis. Soil. Soil samples collected from field before starting and after completion of the experiment followed by drying under shade, crushing and passing through a 2 mm sieve. Processed samples were analyzed for different physicochemical properties of soil viz., pH, organic carbon, available nitrogen, available phosphorus, available potassium and DTPA extractable cobalt. Soil pH was determined by standard procedure (Jackson, 1973). Available nitrogen was estimated by the macro Kjeldahls method (Jackson, 1973). Available phosphorus was determined colometrically by following Vanado-phosphomolybdic acid yellow-color method (Jackson, 1973). Available potassium was determined flame photometrically by standard method (Jackson, 1973). Cobalt was estimated by atomic absorption spectrophotometer (manual supplied by GBC Scientific Equipment Pty Ltd., Australia -GBC 932AA) following DTPA (Di-ethylene Triamine Penta Acetic acid) extraction method (Lindsay & Norvell, 1978).

Plant. Kernels of groundnut were dried at 70°C for 48 hours in an oven. For homogenization, these were ground in a stainless steel grinder and subsequently used for chemical analysis. Processed plant samples were digested in concentrated H_2SO_4 + H_2O_2 on a hot plate under controlled temperature for estimation of N and in triacid (HNO_3 : $HClO_4$: H_2SO_4 :: 10:4:1) mixture for P and K. The modified Kjeldahl method was adopted for analyzing the total nitrogen content. Phosphorus was determined colorimetrically following vanado-phosphomolybdic yellow color method and the potassium flame photometrically, as depicted in Piper (1942). Cobalt was determined by atomic absorption spectrophotometer (Jackson, 1973).

Oil content of the kernel of the peanut was determined by the solvent extraction method using Soxhlet apparatus (AOAC, 1990). Kernels, taken by random selection, were washed with distilled water, oven dried at 60°C to constant weight and crushed with mortar-pestle. Five grams of such crushed material was used for analyzing the oil percentage.

Statistical analysis. The recorded data were analyzed statistically following the standard procedure as described by Gomez and Gomez (1984). Treatment differences were tested at 5% level of significance by *F*-test.

RESULTS AND DISCUSSION

Yield components, kernel yield and oil content. There was a significant influence of different treatments of microbial inoculants and cobalt on yield components, kernel yield and oil content of groundnut (Table 1). Average seed yield of groundnut was about 10.0% higher with 0.21 kg cobalt ha^{-1} over the no cobalt application. However, increasing the level of cobalt to 0.42 kg ha^{-1} did not increase the kernel yield. The lower dose of cobalt helped in better nodulation and consequently a better growth and yield, but at a higher level cobalt reduced the bacterial population in the rhizosphere; as a result, nodulation was hampered which led to lower growth and yield of the crop (Jana & Sounda, 1994). Similarly, seed yield was recorded to be maximum for *Rhizobium* inoculant, which was 16.5% and 10.7% higher over no inoculant and phosphobacterium inoculants respectively. This yield advantage could be attributed to the beneficial role of *Rhizobium* in the N nutrition through nodulation and better growth or development (Salui et al., 1998; Subramaniyan et al., 2000). The data also revealed that there was a significant interaction between the levels of cobalt and inoculants. Kernel yield was estimated to be maximum with *Rhizobium* inoculant receiving 0.21 kg cobalt ha^{-1} and lowest with no inoculants receiving no cobalt. The rate of infection was slower for the no microbial inoculant treatments since the processes of infection and nodule establishment were restricted by low rhizobial numbers. This perhaps led to a smaller amount of nitrogen fixed, which could not meet the demand of the plant, resulting in low yield.

As in kernel yield, different levels of cobalt significantly influenced the oil content of groundnut. Average oil content increased by 5.1% with 0.21 kg cobalt ha^{-1} over no cobalt and enhancing the cobalt level beyond 0.21 kg ha^{-1} did not significantly increase the oil content. The interaction effect between the levels of cobalt and inoculants was significant. Cobalt application improved the uptake of phosphorus, which was responsible for higher oil content. The same observation was reported by Raj & Rao (1996).

Nutrient content. Levels of microbial inoculants and levels of cobalt significantly influenced the N, P, K (%) and cobalt (mg kg^{-1}) content of groundnut kernel at harvest (Table 2). Regarding percent of N content, maximum value was obtained with *Rhizobium* inoculant which was followed by the phosphobacterium inoculant. Nitrogen concentration was 25.0% and 12.2% higher over no inoculant and phosphobacterium inoculant respectively. Sasidhara & Sreenivasa (1994) obtained the same results. The beneficial effect of *Rhizobium* was also observed by Saad et al. (1998) and Naidu (2000). Average N concentration increased by about 8.0% over no cobalt and enhancing the cobalt level beyond 0.21 kg ha^{-1}, did not increase the N concentration. The interaction effect between the levels of cobalt and inoculants was also significant.

Table 1. Effect of cobalt and microbial inoculants on yield components, kernel yield and oil yield of groundnut (Pooled mean of three years).

Treatment combinations	No. of pods plant^{-1}	No. of kernel pod^{-1}	Test weight (g)	Kernel yield (kg ha^{-1})	Oil content (%)
I_0C_0	18.3	1.75	348.0	1127.6	43.1
$I_0C_{.21}$	23.3	1.80	348.1	1256.9	46.1
$I_0C_{.42}$	21.3	1.71	348.1	1203.8	44.2
I_RC_0	26.3	1.82	348.1	1335.1	46.1
$I_RC_{.21}$	30.8	2.01	348.2	1454.2	48.2
$I_RC_{.42}$	28.4	1.88	348.1	1389.8	47.6
I_PC_0	22.3	1.81	348.0	1200.9	45.3
$I_PC_{.21}$	26.4	1.95	348.1	1317.1	47.1
$I_PC_{.42}$	24.1	1.85	348.0	1260.7	46.3
LSD (P=0.05) Inoculant	1.31	0.11	NS	48.73	0.40
Cobalt	1.34	NS	NS	40.12	0.48
Inoculant x Cobalt	1.53	0.15	NS	50.64	0.51

Note: $I_0 = I_0$ = No inoculant, I_R = *Rhizobium* inoculant and I_P = Phosphobacterium inoculant. C_0, $C_{.21}$, $C_{.42}$ = zero dose, 0.21 kg cobalt per ha and 0.42 kg cobalt per ha respectively. NS = Not significant.

The cause of the highest concentration of N in *Rhizobium* inoculant with the lower dose of cobalt (0.21 kg cobalt ha^{-1}) was that it had increased the population of nitrogen-fixing bacteria (*Rhizobium*) in the rhizosphere and the high number of rhizobia available for infection of the root facilitated rapid nodule establishment. Cobalt limitation has been shown to reduce growth and nitrogen fixation in *Lupinus angustifolius* dependent on symbiotically-fixed nitrogen (Riley and Dilworth, 1982). This might be due to the fact that cobalt governs the activity of three specific enzyme systems in *Rhizobium* responsible for nodulation and nitrogen fixation. These are Methionine synthase**,** Ribonucleotide reductase and Methylmalonyl co-enzyme A mutase (Das, 2000). Similar positive effect of cobalt application on nitrogen fixation was confirmed by Yadav et al. (1988).

Potassium concentration also followed the same trend as nitrogen concentration and was 27.1% and 13.0% higher over no inoculant and phosphobacterium inoculant respectively. Application of cobalt at the rate 0.21 kg ha^{-1} increased the K content of kernels by 7.7% over no cobalt.

P concentration did not follow the same trend as N and K. Groundnut seeds inoculated with phosphobacterium culture resulted in 12.2% and 5.5% higher P concentration over control (no inoculant) and *Rhizobium* inoculant respectively. Cobalt applied at the rate 0.21 kg ha^{-1} significantly increased P concentration over the no cobalt application; the extent of increase was about 7.7%. Similar observations regarding the effect of the inoculant and cobalt on nutrient concentration was also reported by Jana & Sounda (1994) and Raj & Rao (1996).

Table 2: Effect of cobalt and microbial inoculants on nutrient concentration of kernel of groundnut (Pooled mean of three years).

Treatment combinations	N (%)	P (%)	K (%)	Co, mg kg^{-1}
I_0C_0	4.07	0.46	0.46	0.01
$I_0C_{.21}$	4.61	0.51	0.49	0.20
$I_0C_{.42}$	4.39	0.49	0.48	0.27
I_RC_0	5.38	0.53	0.58	0.02
$I_RC_{.21}$	5.61	0.57	0.64	0.23
$I_RC_{.42}$	5.42	0.55	0.60	0.38
I_PC_0	4.75	0.56	0.52	0.02
$I_PC_{.21}$	5.12	0.61	0.56	0.31
$I_PC_{.42}$	4.83	0.58	0.53	0.40
LSD (P=0.05) Inoculant	0.12	0.02	0.03	0.057
Cobalt	0.15	0.03	0.02	0.046
Inoculant x Cobalt	0.26	0.02	0.01	0.051

Note: I_0 = I_0 = No inoculant, I_R = *Rhizobium* inoculant and I_P = Phosphobacterium inoculant. C_0, $C_{.21}$, $C_{.42}$ = Cobalt at the rate zero, 0.21 kg ha^{-1} and 0.42 kg ha^{-1} respectively.

Cobalt concentration of kernels followed the same trend as phosphorus and phosphobacterium inoculant had higher cobalt concentration as compared to the *Rhizobium* inoculant. The data also revealed that there was a significant interaction between the levels of cobalt and the inoculants. Phosphobacterium inoculant along with cobalt at the rate 0.42 kg ha^{-1} gave the maximum cobalt concentration. The concentration of cobalt in kernels under cobalt application at the rate 0.21 kg ha^{-1} did not cross the toxic limit. There are reports on the toxicity levels of cobalt, where values vary from 0.4 mg kg^{-1} (dry weight basis) in clover to a few milligrams per kilogram dry weight in bean and cabbage (Das, 2000).

Nutrient uptake. As in nutrient concentration, nutrient uptake by the kernel responded to application of cobalt (Fig. 1). Nitrogen uptake increased under two levels of cobalt application over without cobalt application. The extent of increase over the no cobalt application was 18.3% and 9.6% with cobalt applied at 0.21 kg ha^{-1} and 0.42 kg ha^{-1} respectively. On an average the nitrogen uptake with *Rhizobium* inoculant was 46.6% and 23.0% higher than no inoculant and phosphobacterium inoculant respectively. *Rhizobium* inoculant increased infection of roots with rhizobia and thereby increased nodule formation resulting in greater nitrogen fixation. Higher nitrogen fixation led to greater nitrogen uptake. Similarly phosphobacterium inoculant solubilized the unavailable phosphorus to plant-available form, and thereby increased phosphorus uptake. The interaction effect between the levels of cobalt and inoculants with respect to nitrogen uptake was significant. This might be due to the fact that *Rhizobium* and other nitrogen-fixing organisms have an absolute cobalt requirement (Das, 2000).

Application of cobalt had significant effect on phosphorus uptake. On average, the uptake of P increased by 18.44% and 11.02% with application of cobalt applied at 0.21 kg ha^{-1} and 0.42 kg ha^{-1}, respectively over the no cobalt application. The average P uptake significantly varied. *Rhizobium* inoculant gave the maximum P uptake with 31.4% and 4.6% higher value than no inoculant and phosphobacterium inoculant respectively. As in nitrogen, there was a significant interaction effect of the levels of cobalt and inoculants on P uptake by groundnut kernels. Mane & Rout (1993) reported higher P uptake by groundnut plant in *Rhizobium* inoculant.

Likewise, *Rhizobium* and phosphobacterium inoculants had about 47.9% and 18.9% higher K uptake than no inoculant. As in the case of N and P, the interaction effect of the levels of cobalt and inoculants was also significant with respect to potassium uptake. *Rhizobium* inoculant with 0.21 kg cobalt ha^{-1} as well as 0.42 kg cobalt ha^{-1} had the maximum uptake of K as against the lowest with no inoculant without cobalt application. Uptake of major nutrients increased with the lower level of cobalt application; higher nutrient availability and yield were presumably responsible for higher accumulation of the nutrients in question.

Application of cobalt had significant effect on cobalt uptake. On average, uptake of cobalt increased by 36.3% when the application dose of cobalt was increased from 0.21 to 0.42 kg cobalt ha^{-1}. But there was no significant difference between cobalt uptake by *Rhizobium* and phosphobacterium inoculants and it was 50% higher than control.

Residual soil fertility. The use of different microbial inoculants and cobalt improve the residual soil fertility in terms of available N, P, K and Co after two years (Fig. 2). After two years the nitrogen content of the soil increased from an initial value of 103 mg kg^{-1} to as high as 133 mg kg^{-1} under application of *Rhizobium* inoculant. At the maturity stage of groundnut, nodules degraded and fixed nitrogen added to the soil leading to improvement in nitrogen content of the soil. The build-up of P was also at maximum under application of phosphobacterium inoculant with an increment in average phosphorus content of the soil up to 28 mg kg^{-1} from an initial value of 20 mg kg^{-1}. Phosphobacterium inoculant increased the available phosphorus status of the soil by solubilizing unavailable phosphorus to available form. Cobalt application improved the cobalt accumulation in soil. Increasing the dose of cobalt subsequently increased the available cobalt content of the soil with a maximum value of 0.31 mg kg^{-1}. Improvement of the cobalt content of the soil was higher in association with phosphobacterium inoculant than in combination with *Rhizobium* inoculant.

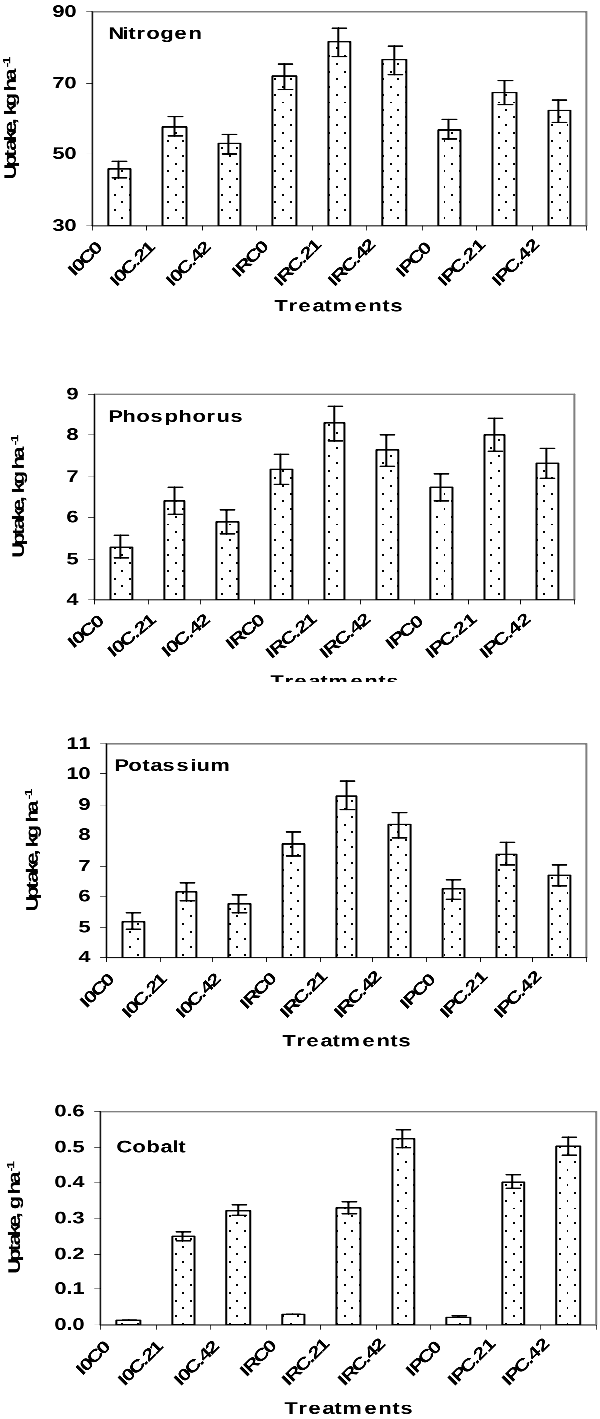

Fig. 1. Effect of cobalt and microbial inoculants on nutrient uptake by kernel of groundnut (Pooled mean of three years).

Note: I_0 = No inoculant, I_R = *Rhizobium* inoculant and I_P = Phosphobacterium inoculant. C_0, $C_{.21}$, $C_{.42}$ = Cobalt applied at zero, 0.21 and 0.42 kg ha^{-1} respectively.

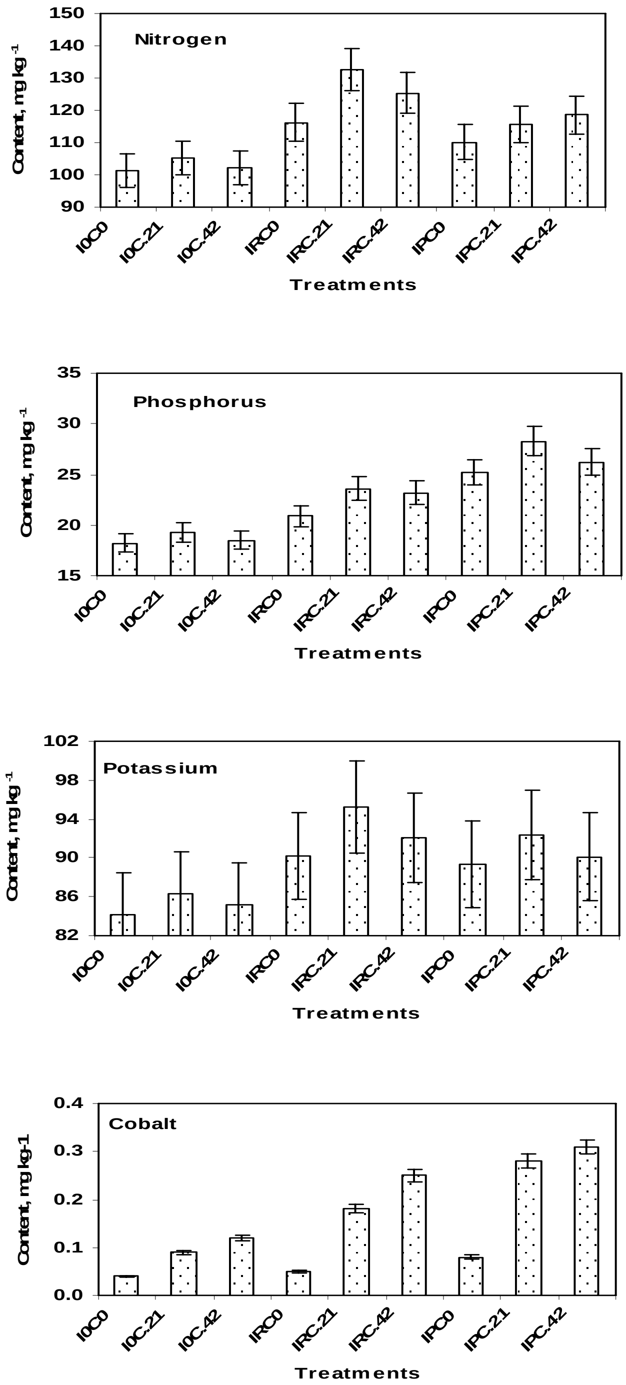

Fig. 2. Effect of different levels of cobalt and microbial inoculants on available N, P, K and Co content of soil after three years of experiment.

Note: I_0 =No inoculant, I_R = *Rhizobium* inoculant and I_P = Phosphobacterium inoculant. C_0, $C_{.21}$, $C_{.42}$ = Cobalt applied at zero, 0.21 and 0.42 kg ha^{-1} respectively.

Response Curve. The relationship between levels of input and corresponding output indicates response function or production function. A response curve is formed when the output is related to variations in the level of a single factor. In order to study the relationship between levels of cobalt and kernel yield of groundnut crop, a response curve was fitted. The following response equation was used for yield-dose relationship:

$\delta = \alpha + \beta x + \gamma x^2$ (quadratic response)
where, δ = Kernel yield of groundnut in kg ha^{-1}
x = Level of dose of cobalt in kg ha^{-1}
α, β and γ are constants

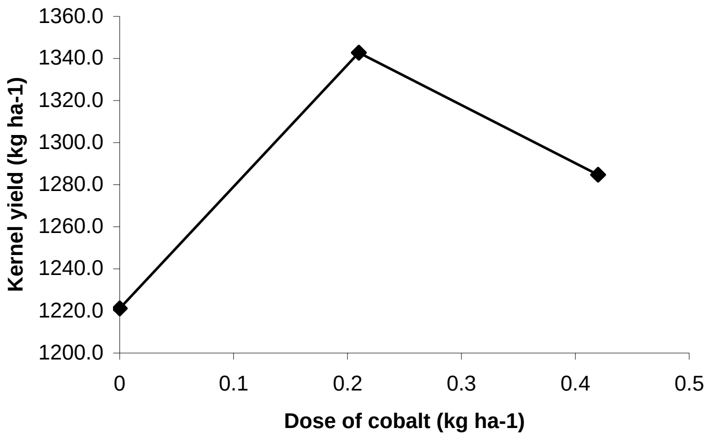

Fig. 3. Response curve of kernel yield of groundnut to cobalt application.

The response of cobalt application to groundnut was found to be quadratic in nature (Fig. 3). The response equation is given below:

$\delta = 1221.73 + 1006.1x - 2035.1x^2$
Where, δ = Kernel yield of groundnut in kg ha^{-1}
x = Levels of cobalt in kg ha^{-1}

The Profit Maximizing Rate (PMR) of cobalt was found to be 0.19 kg ha^{-1} for groundnut crop with an optimum yield augmentation of 9.6%, but the Yield Maximizing Rate (YMR) of cobalt was found to be 0.27 kg ha^{-1} for groundnut crop with a yield increase of 10.1% with respect to without cobalt application.

CONCLUSIONS

It can be concluded from the above discussion that the application of *Rhizobium* or phosphobacterium inoculant increased the yield and nutrient uptake of groundnut as compared to no inoculant. A lower dose of cobalt resulted in better performance of the crop as compared to the higher dose. Combined application of *Rhizobium* or phosphobacterium inoculant and cobalt was found to be beneficial over a single application of either inoculant or cobalt. Besides the yield advantage, residual soil fertility in terms of available N, P, K and Co was also improved under the combined application of microbial inoculants and cobalt as compared to sole application of only an inoculant or cobalt.

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